Forest Research Report No. 112

# Poplar Clonal Variation in Frost Hardiness and Electrical Impedance During the Fall

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Ontario Ministry of Natural Resources

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ISSN 0301-3924
ISBN 0-7729-0942-3

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Printed in Ontario, Canada

Additional copies of this publication may be obtained from:

Ontario Tree Improvement and
Forest Biomass Institute
Ministry of Natural Resources
Maple, Ontario L0J 1E0
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Pelkonen, P. and C. Glerum. 1985. Poplar clonal variation in frost hardiness and electrical impedance during the fall. Ontario Ministry of Natural Resources, Forest Research Report No. 112. 12 p.

## ABSTRACT

Three trials were conducted in the fall and winter of 1982 to determine the usefulness of electrical impedance in concert with freezing tests for determining the frost hardiness of eight poplar clones. Impedance increased during the fall with increasing frost hardiness and the impedance increase was somewhat greater in the frost tolerant clones than in the frost susceptible ones. Post freezing 1 kHz impedance and the 1 kHz/1 MHz ratio showed a clear decrease due to injury. Impedance is not yet suitable for routine measurements of frost hardiness without the accompanying freezing tests.

## INTRODUCTION

Frost hardiness should be a selection criterion in any tree breeding program that develops fast growing varieties for cold climates (Glerum 1976). During the last few decades fast growing progeny and clones have been successfully selected and bred in various countries. However, the screening of forest trees for frost hardiness has been slow, being dependent on time consuming and expensive field trials, due to the lack of a rapid direct method.

Electrical impedance has been used in many investigations as a rapid non-destructive method for determining freezing injuries and winter hardiness in the tissues of woody plants (Glerum 1962, Wilner 1962, Glerum 1970, van den Driessche 1973, Timmis 1976, and Glerum 1980). Although the sensitivity of impedance to variations in stem diameter and temperature has made the use of this method on a practical scale difficult, it has been suggested that electrical impedance could assist in the screening of progeny and clones in breeding trials (van den Driessche 1973). Fast growing deciduous trees such as poplar, are well suited to impedance investigations because they coppice abundantly and produce many branches that increase in diameter quickly to a measurable size. Many poplar species also have good rooting ability, which makes clonal replication for impedance testing feasible. These characteristics also facilitate the study of impedance in association with greenhouse survival tests.

The objective of the present investigation was to study the application of electrical impedance techniques together with freezing tests in assessing frost hardiness properties of several poplar clones.

## MATERIALS AND METHODS

During the 1981 fall and early winter period three experiments were conducted using current-year coppice sprouts of eight different poplar clones (Table 1) grown in stool beds of the Ontario Tree Improvement and Forest Biomass Institute at Maple (Lat. N43°51', Long. W79°31').

TABLE 1 List of clones, their geographical origins (Ronald 1982) and the mean height of the four tallest coppices in the stool beds during the fall, 1981.

| Clone | Systematic origin | Sex | Geographic origin | Height (cm) |
|---|---|---|---|---|
| DN30 | *deltoides* x *euramericana* cv. Canada Blanc | | France | 220 |
| DN22 | *deltoides* x *euramericana* cv. "I 262" | | Italy | 260 |
| D155 | *deltoides* var. *occidentalis* (pop. 645) | | Marius River, Montana | 210 |
| D203 | *deltoides* var. *occidentalis* (pop. 641) | | Red River, Manitoba | 250 |
| TAC8 | *tacamahaca* | M | Beverly Swamp, Ontario | 220 |
| TAC19 | *balsamifera* | M | Flesherton, Ontario | 180 |
| TAC9 | *tacamahaca* 56 | F | Winnipeg, Manitoba | 150 |
| TAC38 | *balsamifera* | | Timmins, Ontario | 190 |

Experiment 1

The purpose of this experiment was to observe the electrical impedance and frost hardiness trends of the various clones during the fall.

Fifteen cuttings of each clone were collected from the stool beds once a week from October 5 to November 23, 1981. In addition, one sample was taken in the winter, on January 18, 1982. These cuttings were subjected to three previously designated over-night freezing test temperatures, which varied from -6° to -40°C, being higher at the beginning and lower at the end of the testing period.

The cuttings from each clone were divided in groups of five and after electrical impedance had been measured they were put into plastic bags (i.e. 5 cuttings/bag and 3 bags/clone). Subsequently they were placed into a freezing chamber for freezing at three different temperatures. Thermocouples were inserted among and into the cuttings for monitoring the temperatures during freezing. The rate of temperature decrease below 0°C varied from 2 to 6 degrees per hour, depending on the designated minimum temperature. After the minimum temperature had been reached, the cuttings were removed from the freezing unit and allowed to warm up gradually in an insulated box to 4°C.

Due to the effect of diameter on electrical impedance, cuttings with diameters greater than 7 mm were used whenever available. However, because of insufficient plant material some smaller cuttings had to be used and clones TAC9, TAC19 and TAC38 were less intensively sampled than the other five clones.

Experiment 2

The purpose of this experiment was to observe the effects of constant temperatures on the electrical impedance and frost hardiness of the clones during the fall.

Five cuttings of each clone were collected on October 13 and subjected to constant temperatures of -11, +4 and +20°C for various days until December 3 and the cuttings were subjected three times to overnight freezing at -18°C (2X) and -30°C (Fig. 10D). Electrical impedance was measured 10 times during the course of this experiment. The cuttings were rolled in wet paper towels and kept in plastic bags during the course of the experiment, except when impedance was measured.

Experiment 3

The purpose of this experiment was to observe the changes in the electrical impedance trends when cuttings go from a dormant and frost hardy state to an actively growing and nonhardy state.

Five cuttings of clones TAC8 and DN22 were collected on December 16 and kept at room temperature until January 26, 1982. The electrical impedance was measured 20 times during this period. The cuttings were rolled in wet paper towels and kept in a plastic covered beaker which contained distilled water.

## Electrical Impedance Measurement Procedures

Impedance measurements in experiments 1 and 2 were taken with a Wide Range Impedance Bridge (Glerum 1970), using electrodes made from steel needles 7.5 mm long, 1 mm in diameter, and spaced 15 mm apart. In experiment 3 a prototype of a Finnish impedance device, but with similar electrodes, was used.

Impedance was measured just before and after freezing at 1 kHz and 1 MHz in the laboratory when the tissue temperatures were at ambient, which was reached from 1/2 to 1 hour after the freezing test was completed.

To compensate for the diameter effect, electrical impedance was adjusted for diameter by the following equation:

$$Z = (D/a)^{-3} + b, \qquad [1]$$

where: Z = electrical impedance (in kilo ohms)
D = cutting diameter in mm
a = a constant, which determines the curvature of the line
b = y intercept, i.e. Minimum Magnitude of Impedance

## Greenhouse Growth Test

Immediately after completion of the freezing tests and impedance measurements, the cuttings of experiment 1 were planted in plastic containers with a sand/peat mixture (2:1 V/V) and placed in a greenhouse under natural light conditions. Watering and fertilization was done by hand as needed. Bud flushing and new shoot elongation were measured twice a week, with the final assessment in the beginning of March, 1982.

# RESULTS

## Experiment 1

Electrical impedance usually decreased with increasing cutting diameter but the diameter effect was negligible above 7-8 mm (Fig. 1). The variation in both 1 kHz and 1 MHz impedance due to diameter was slight for cuttings with diameters greater than 7-8 mm. The relationship between electrical impedance and stem diameter varied over the period of testing, being most pronounced during early fall.

The seasonal change in 1 kHz and 1 MHz impedance is presented for five clones (3 clones were not sampled every week) in Fig. 2. The impedance has been adjusted for diameter effect with equation 1. For all clones, the electrical impedance tended to increase towards the end of the fall to a high on November 23. In winter, the 1 kHz impedance for three clones decreased but remained high for two others. The highest 1 kHz impedance was measured in clones DN22 and DN30, while the lowest impedance was in clone D203. The 1 MHz impedance showed proportionately similar trends as for the 1 kHz impedance.

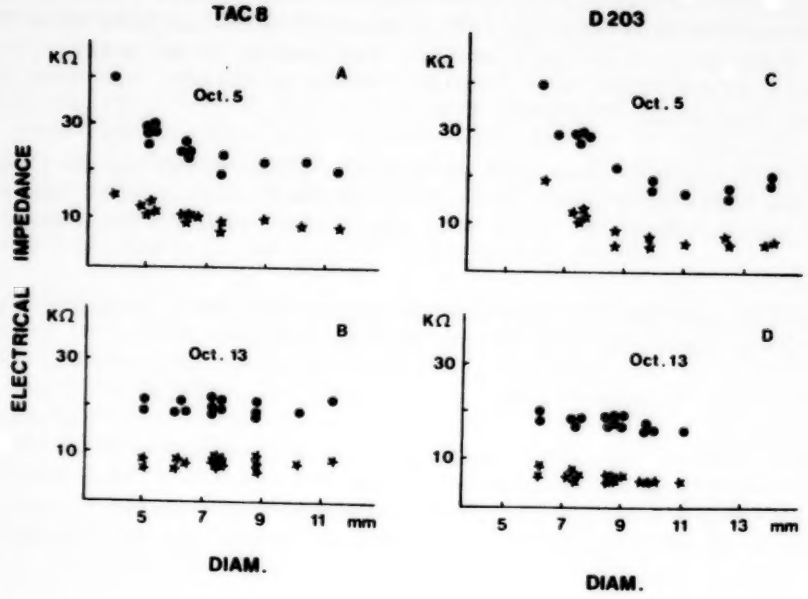


Figure 1 Influence of diameter on 1 kHz (●) and 1 MHz (★) impedance for two clones in two different weeks.
A) TAC8 on Oct. 5; B) TAC8 on Oct. 13; C) D203 on Oct. 5;
D) D203 on Oct. 13.

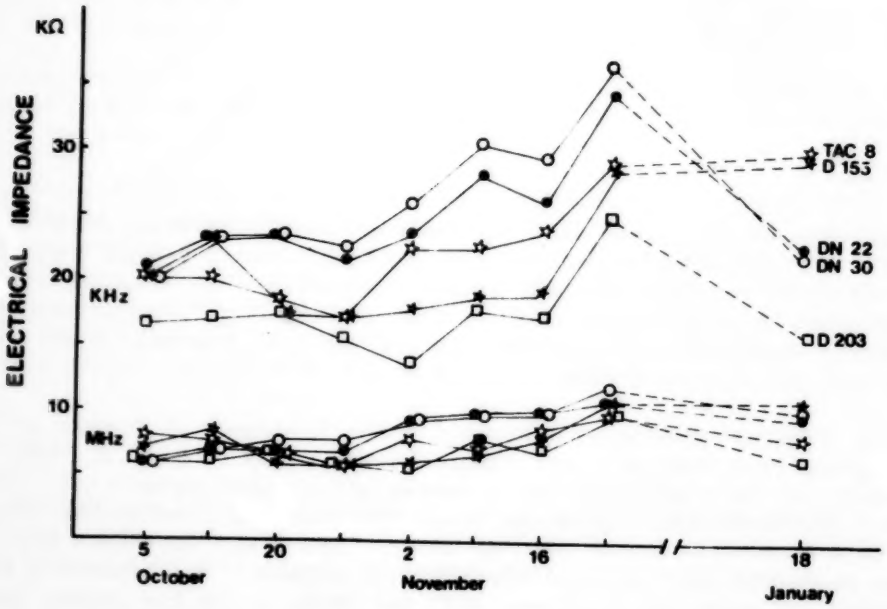


Figure 2 The 1 kHz and 1 MHz post-freezing impedance of five different clones from Oct. 5 to Jan. 18. Each point is the average of 5 cuttings per clone. Diameter effect has been compensated for by using equation (1).

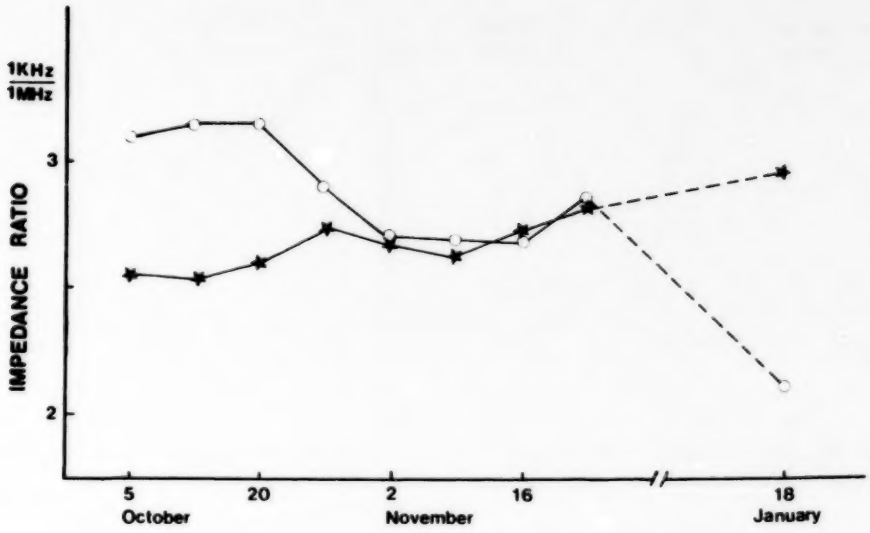


Figure 3 The average 1 kHz/1 MHz impedance ratio in clones DN22, DN30, D203 (o) and TAC8, TAC9, TAC19, TAC38, D155 (★) during the period of testing.

The eight clones were divided on the basis of their 1 kHz/1 MHz impedance ratio into two groups (Fig. 3). Over the period of testing the average ratio decreased in the frost susceptible clonal group of DN22, DN30 and D203 while it increased slightly in the frost tolerant TAC8, TAC9, TAC19, TAC38 and D155 group. The low impedance ratios indicated damage for DN22, DN30 and D203 which was directly correlated with the poor survival in the greenhouse test, especially for D203, towards the end of the experiment.

During the first seven weeks of testing (from Oct. 5 to Nov. 16) one set of cuttings from each clone was frozen at -18°C. The differences in impedance before and after freezing at 1 kHz and at 1 MHz for the clones is shown in Fig. 4. Because changes in impedance are related to prevailing temperatures in the field, the differences in impedance have been plotted over the mean of the minimum and maximum temperatures of the four days preceding the freezing treatment. There were marked decreases in the impedance differences for the first few weeks but by the seventh week, when field temperatures were lowest, the differences approached zero in every clone after freezing, indicating that -18°C had caused no damage. The trend in the changes were similar for both 1 kHz and 1 MHz.

In general, the post-freezing changes in impedance characteristics were slight in the hardiest clones (TAC8, TAC9, TAC19, TAC38, and D155), while they showed considerable variation in the frost susceptible clones (DN22, DN30 and DN203).

Changes in the 1 kHz/1 MHz ratios before and after freezing exhibited greater variability between the cuttings of the frost susceptible clones than those of the frost tolerant clones (Fig. 5), especially in the beginning of the testing period.

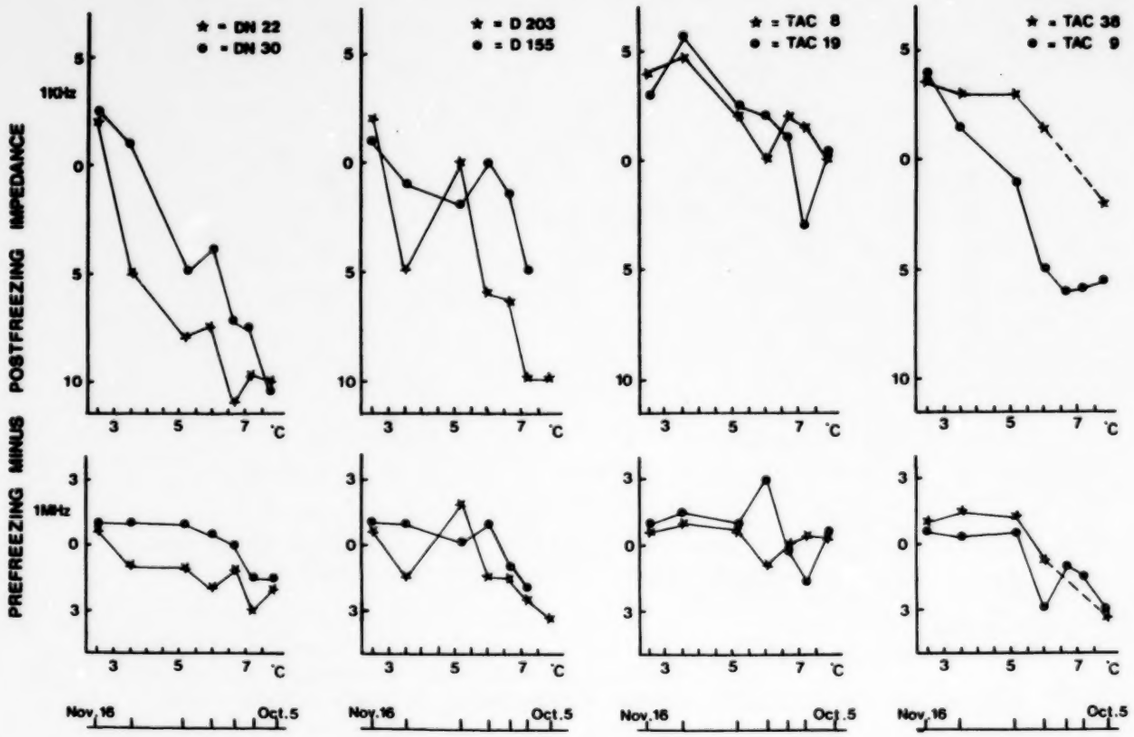


Figure 4 The difference between pre- and post-freezing impedance at 1 kHz and at 1 MHz in the clones as a function of mean field temperature (Tmin+Tmax)/2 of the 4 days preceding the freezing at -18°C.

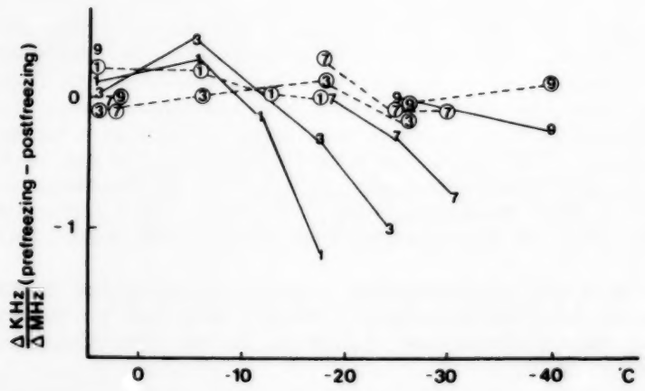


Figure 5 Differences between pre- and post-freezing 1 kHz/1 MHz in clones, DN30 (uncircled) and TAC19 (circled). Numbers refer to measuring week. Oct. 5 (1), Oct. 20 (3), Nov. 16 (7) and Jan. 18 (9).



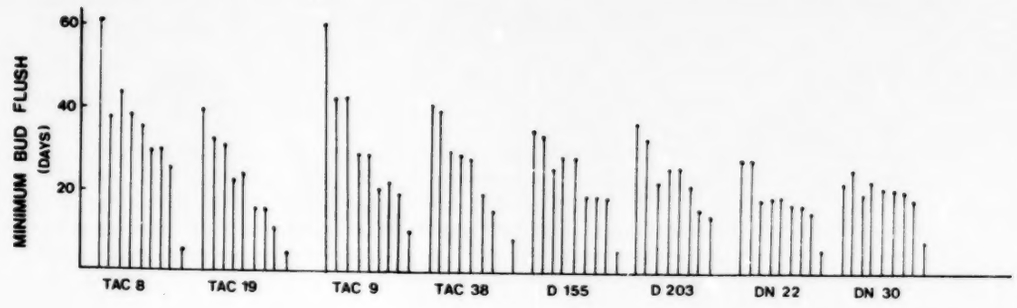


Figure 6 The number of days needed for the first three buds to flush on cuttings in the greenhouse after the freezing treatments (Test 1 to 9).

After the freezing test the buds on the cuttings needed from 5 to 50 days to flush in the greenhouse (Fig. 6). The time required for bud flush was less variable as well as shorter for the deltoides clones than for the TAC clones, especially in the beginning of the test period. This indicates that there are considerable differences in dormancy requirements for the various clones.

The survival of cuttings after the freezing tests was not only dependent on freezing injury but also on the length of time required for bud flush. This was particularly noticeable in October when the hardiest clones (TAC8, TAC9, TAC19, TAC38 and D155), required more time for bud flush than the frost susceptible clones (Fig. 6). These hardy clones had poor survival (Fig. 7) in October due to the confounding effect of the dormancy requirements. The freezing treatment at lower temperatures, improved the survival in these clones.

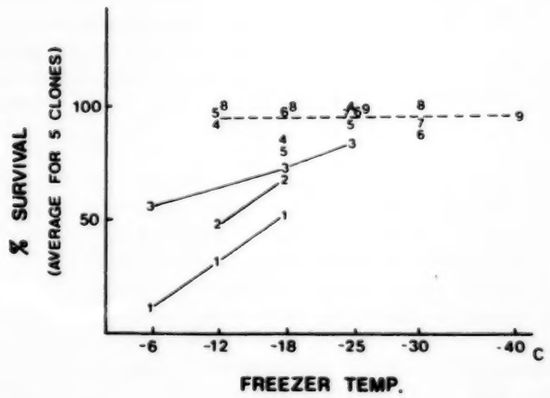


Figure 7 The effect of freezing on survival (average of 25 cuttings) of the frost-tolerant clones (TAC8, TAC9, TAC19, TAC38, D155). The numbers refer to the week of testing from October 5 (1) to November 23 (8) and January 18 to 25 (9).

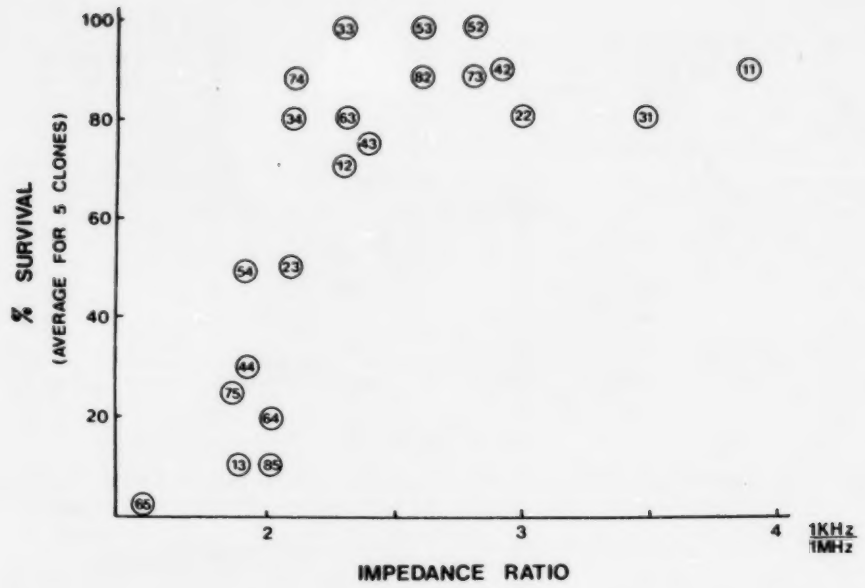


Figure 8 The relationship between survival and the average 1 kHz/1 MHz impedance ratio of the cuttings for the frost susceptible clones (DN22, DN30 and D203). The first number in the circle refers to week of testing and the second one to freezing temperatures of -6, -12, -18, -25, -30°C as 1 to 5 respectively.

Freezing at the lowest temperatures (-30°C) decreased survival of the frost susceptible clones (DN22, DN30 and D203) and was associated with low impedance ratios (Fig. 8). Survival varied weekly but by October 20 (week 3) most of the cuttings from these clones survived freezing at -18°C. The survival of the frost susceptible clones was 70% or higher when the average 1 kHz/1 MHz ratio was greater than 2.1 while a low ratio of 1.5 indicated fatal damage. The critical range of the impedance ratio for injury prediction varied from 1.8 to 2.2.

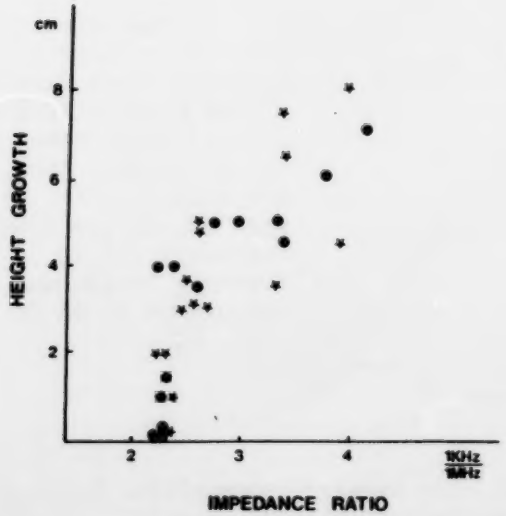


Figure 9 The relationship between height growth and post-freezing impedance ratio of clones DN22 (●) and DN30 (★) that were subjected to freezing in the third week (Oct. 20).

The impedance ratio was also indicative of the vitality of the cuttings. Those cuttings that had a low 1 kHz/1 MHz ratio also had low subsequent height growth in the greenhouse (Fig. 9).

Experiment 2

During the first days of this experiment, there were no significant changes in the 1 kHz impedance between the frost tolerant (TAC8, TAC9, TAC19, TAC38 and DN155) and frost susceptible (DN22, DN30 and D203) clones, but a pronounced clonal difference was observed on October 28 after the cuttings were subjected to -11°C for five days followed by -18°C for one night (Fig. 10). During this six day period (Oct. 22 to Oct. 28)

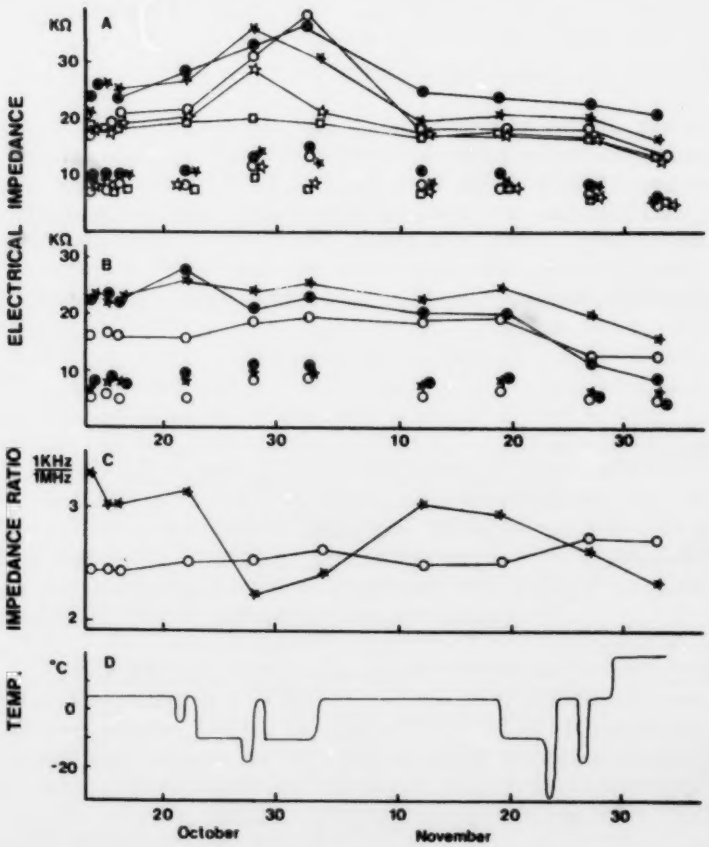


Figure 10 Changes in impedance characteristics during the laboratory experiment no. 2 from October 13 to December 3.

A. The 1 kHz impedance (lined) and the 1 MHz impedance (unlined) offrost tolerant clones (5 cuttings/clone) TAC8 (o), TAC9 (□), TAC19 (☆), TAC38 (★), and D155 (●).

B. As A. but of frost susceptible clones DN22 (●), DN30 (★), and D203 (o).

C. The average 1 kHz/1 MHz impedance ratio of the frost tolerant clones in A (o) and the frost susceptible clones in B (★).

D. The temperature to which the cuttings were exposed during the course of the experiment.

the 1 kHz impedance increased in the frost tolerant clones while it remained relatively unchanged in the frost susceptible ones. Similarly clonal differences were also observed after the second period of freezing (Nov. 19 to 27) when the lowest temperature reached was -30°C and little to no reduction in 1 kHz impedance occurred in the frost tolerant clones but there was a definite reduction in the frost susceptible clones. There was relatively little change in the 1 MHz impedance during the course of the experiment.

Pronounced clonal differences were also exhibited in the 1 kHz/1 MHz ratio. In the frost tolerant clones little change occurred in this ratio after the freezing periods, but the frost susceptible clones showed significant reductions. The low ratios indicated damage and the lowest ratio of 1.6 was measured in DN22 cuttings and not one of these showed any signs of life in the subsequent greenhouse vitality test.

Experiment 3

The 1 kHz impedance (Fig. 11) for both clones decreased rapidly prior to bud flush but it had levelled off at time of bud flush, which occurred in 11 days for DN22 and in 12 days for TAC8. The trend for the 1 MHz impedance was similar to that of the 1 kHz impedance, but considerably lower in magnitude. The impedance ratio increased somewhat prior to bud flush for both clones but the ratio of the frost susceptible clone DN22 was considerably higher than that for the frost tolerant clone TAC8.

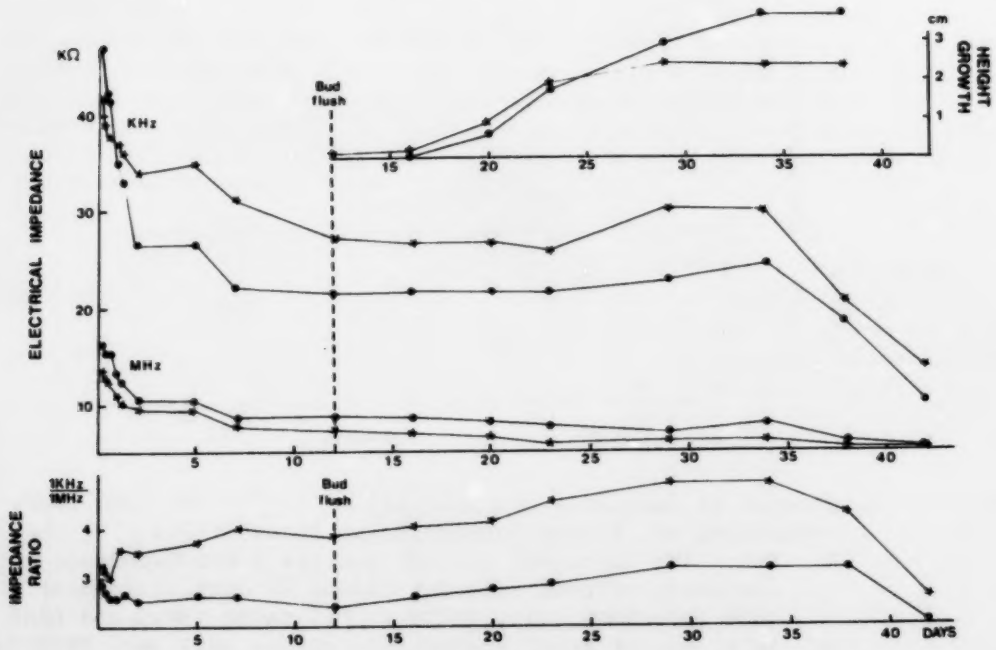


Figure 11 Changes in impedance characteristics in relation to growth of cuttings from frost tolerant clone TAC8 (●) and frost susceptible clone DN22 (★) over a period of 40 days when the cuttings were kept at room temperature.

## DISCUSSION

There were differences in the before and after freezing impedance characteristics for the eight clones during the period of testing (Oct. 5 to Jan. 18). The response of clones from France (DN30) and from Italy (DN22) to changes in weather conditions as well as to freezing treatments was similar (Fig. 2), but the electrical impedance characteristics and the postplanting vitality assessment suggested that DN30 was more frost-tolerant than DN22. The changes in impedance of the deltoides clone (D203) were similar to those in the DN clones. However, the northerly geographical origin of clone D203 (Red River, Manitoba) suggests that it should have greater frost-tolerance than was observed in this study. Both the impedance characteristics and the vitality assessments indicated frost-susceptibility but we suspect that the poor performance of D203 was due to a disease, which decreased its vitality and survival. In a field assessment on September 10, 1981, all the leaves of clone D203 were affected by rust, while about 80% of the TAC9 leaves and 60% of D155 leaves were affected. There were no signs of disease in the other clones.

The deltoides clone D155 (Marius River, Montana) was more frost-tolerant than the DN clones and D203 but its impedance was almost the same in late November as by mid-January, while it decreased drastically in the less tolerant clones. It is possible that some of the variability in the impedance of D155 was due to the rust damage.

The tacamahaca (TAC8 and TAC9) and balsamifera (TAC19 and TAC38) clones were frost-tolerant and the TAC9 clone was the hardiest in spite of its heavy rust damage. The impedance characteristics of these clones changed only slightly even after freezing tests at the lowest temperature. Freezing at -40°C in mid-January increased the 1 kHz/1 MHz impedance ratio of these clones only slightly. These slight changes suggest that the cuttings were able to withstand considerably lower temperatures by mid-winter. The results of the vitality tests in the greenhouse were in full agreement with the changes in impedance characteristics, but only later in the fall, because in early October, these frost-tolerant clones resumed growth in the greenhouse slowly because of the confounding effect of their dormancy requirements.

Changes in the 1 kHz or 1 MHz impedance were not only related to freezing test temperatures but also to prevailing temperatures in the field or in the laboratory prior to freezing. With decreasing temperatures in the field the impedance of poplar stem tissues increased towards the end of the fall. This type of autumnal trend has been reported for various other woody plants (Wilner 1962, van den Driessche 1973, Timmis 1976, and Glerum 1980).

Pre-freezing impedance was a poor indicator for post-freezing survival of the poplar cuttings. Fatal injuries due to freezing could best be identified on the basis of the 1 kHz/1 MHz impedance ratio (<2) or the decrease in kHz impedance. Similar observations for various other tree species have been found by other investigators (Glerum 1970, van den Driessche 1973, and Blake 1981). Slightly higher ratios of 2.2 to 2.4 might indicate injury in a frost susceptible clone but not in a frost-tolerant clone. In general, post-freezing 1 kHz impedance as well as the 1 kHz/1 MHz ratio showed a clear decrease due to injury while the magnitude of 1 MHz impedance remained almost unchanged.

The measurements of impedance characteristics in concert with freezing treatments provided an accurate and rapid method for determining the frost hardiness of poplar clones. Impedance is influenced by several factors such as tissue diameter and temperature (Glerum and Krenciglowa 1970), but with proper experimental design, these sources of variation can be minimized. Although, impedance is not yet suitable for routine measurements of frost hardiness, it does provide a useful tool for tree breeders in assessing for frost resistance.

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